facilitate this first purification of the desired target with fluid access to binding buffer, used in conjunction with the capture beads. In this embodiment, capture beads and binding buffer are mixed with the sample in lysis buffer after the cells or viruses are disrupted by mechanical and/or chemical means. In general, the capture beads are magnetic to facilitate handling, although as will be appreciated by those in the art, other systems may use non-magnetic beads, such as polystyrene or silica beads (for example, beads may be captured in a zone by size or on an affinity column).

[0141] The capture beads are coated with a functionality that facilitates capture of the target analytes. For example, for the capture of nucleic acids, the beads can be coated with a negatively charged coating to facilitate the adsorption of positively charged nucleic acids to the surface, which are then washed with buffer, optionally transported on the substrate and then treated with elution buffer to remove the purified nucleic acids for further handling. As will be appreciated by those in the art, there are a number of commercially available bead systems, such as MagaZorb® Beads from Promega, MagMax from Life Tech, or beads from Qiagen, MoBio, BioRad, etc.

[0142] Alternatively, capture beads may be functionalized with capture nucleic acid probes in order to either specifically or non-specifically pull out nucleic acids. For example, the beads may be functionalized with random 6-mers, to generally pull out nucleic acids, or with capture probes specific to the desired target nucleic acids. In some cases, for example when mRNA is the target, beads coated with poly-T capture probes can be used.

[0143] As described below, the beads with the captured target analytes are generally mixed and washed prior to elution of the target analytes from the beads to begin the assay process. As part of this process, beads bound with the target analytes are manipulated using magnets and electrowetting to remove residual fluids and/or amplification inhibitors prior to target elution.

Reagent Zone

[0144] Once the target analytes have been eluted and thus released from the beads, the sample containing the target analytes is then ready for amplification (in the case of nucleic acid assays, or other reactions as necessary for other analytes such as proteins).

[0145] Droplets of sample are dispensed into the reagent zone, which optionally have dry or solid reagents at specific locations on the grid. No particular dispenser structure is required in this step, as the elution volume is split into a desired number of droplets using electrowetting. For instance, if the elution volume is 6 μl and each PCR reaction requires a 1 μl droplet, then three 1 μl droplets can be "pinched off" in a consecutive fashion. As will be appreciated by those in the art, the form of the reagent will depend on the reagent. Some reagents can be dried or in solid form (for example when particular buffers are to be used), others can be lyophilized, etc. Particularly useful embodiments utilize dried reagents with added stabilizers, such as salts, sugars, polysaccharides, polymers or proteins such as gelatins, etc. as will be appreciated by those in the art. For example, Biomatrica produces commercial stabilizers for use in the present system.

[0146] As will be appreciated by those in the art, if used, the dried reagents can be rehydrated in one of two general ways. Either liquid from the LRM is introduced at the appropriate pad or the sample itself serves as an aqueous solvent to put the solid reagents into solution. For example, the appropriate resuspension buffer (which can be water, in some cases) can be added through the top plate from the LRM to a particular pad to rehydrate the reagent(s), and then the reagent droplet can be merged with the sample droplet. Alternatively, the drops containing the target analyte (for example, in elution buffer used to liberate the target analytes from the capture beads) may be transported to a pad containing the dried reagent(s), which are then suspended in the drop itself. One benefit of this embodiment is that the ultimate volume of a droplet does not increase significantly, as it does when a drop of reagent is merged with a drop of sample. This may be particularly useful in situations where multiple reagent additions are required.

[0147] As shown in the Figures, a number of embodiments for nucleic acid amplification and detection include a plurality of pads containing dried reagents. See for example FIG. **33**.

[0148] The number, type and quantity of the different reagents will depend on sample, the target analyte and the desired reaction. For example, for nucleic acid target sequences in a standard PCR reaction, when the starting sample is DNA, the on-board dried reagents include RT-PCR buffer, PCR enzyme (e.g. a Taq polymerase), dNTPs, PCR primers, exonuclease, signal probes, signal buffer and detection buffers (with the lysis buffer, the binding buffer, the elution buffer, the (optional) reconstitution buffer(s), and magnetic bead suspension all being contained in the LRM rather than dried on the substrate). Several specific embodiments are outlined below. However, as will be appreciated by those in the art, any number of configurations of dried reagents and liquid reagents in the LRM can be used.

[0149] The chamber formed from the "bottom" substrate and the top plate, more fully described below, is generally filled with a fluid in which the target analyte drops (usually aqueous solutions) are immiscible, and this immiscible fluid is generally less polar than the solution of the drop. As described in U.S. Pat. No. 8,541,177, columns 60-63, there are two general ways of isolating drops on pads including filling the chamber with an immiscible fluid including immiscible liquids and immiscible gases, or by using the immiscible liquid as a droplet encapsulant, for example giving the droplet a shell of oil by passing the droplet through an air/oil interface, with the former generally being preferred.

[0150] Particularly suitable immiscible fluids for use in the nucleic acid detection assays described herein include, but are not limited to, silicone oils, mineral oil, fluorosilicone oils; hydrocarbons, including for example, alkanes, such as decane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane; aliphatic and aromatic alkanes such as dodecane, hexadecane, and cyclohexane, hydrocarbon oils, mineral oils, paraffin oils; halogenated oils, such as fluorocarbons and perfluorocarbons (e.g. 3M Fluorinert liquids) as well as mixtures of the above. Examples of suitable gas filler fluids include, without limitation, air, argon, nitrogen, carbon dioxide, oxygen, humidified air, any inert gases. In one embodiment, the primary phase is an aqueous solution, and the secondary phase is air or oil, which is relatively immiscible with water. In another embodiment, the filler fluid includes a gas that fills the space between the plates surrounding the droplets. A preferred filler fluid is low-viscosity oil, such as silicone oil. Other suitable fluids are described in U.S. Patent Application No. 60/736,399, entitled "Filler Fluids for Droplet-Based Microfluidics" filed on Nov. 14, 2005, the